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Effect of Different Extraction Methods on Phytochemical Profile, Total Flavonoid and Phenolic Contents, and Antioxidant Activity of *Cajanus cajan* Seed Extracts

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Abstract

Cajanus cajan (pigeon pea) seeds are considered a promising source of natural antioxidants; however, limited information is available regarding the influence of extraction methods on their phytochemical composition and antioxidant properties. This study aimed to evaluate the effect of different extraction methods on the phytochemical profile, functional groups, total flavonoid and phenolic contents, and antioxidant activity of *Cajanus cajan* seed extracts. Ethanol maceration and water soaking were used as conventional extraction methods. Phytochemical screening confirmed the presence of flavonoids, alkaloids, steroids/terpenoids, saponins, and tannins in both extracts. Fourier-transform infrared spectroscopy analysis revealed hydroxyl, carbon–carbon double bond, and carbon–oxygen functional groups associated with bioactive compounds. The ethanol extract showed a higher flavonoid content (1.519±0.104 mg QE/g), while the aqueous extract exhibited a higher phenolic content (6.373±0.059 mg GAE/g). Antioxidant analysis using a radical scavenging assay indicated stronger activity in the ethanol extract, with lower inhibitory concentration values compared to the aqueous extract (39.814±3.523 ppm and 222.296±30.120 ppm, respectively). These findings demonstrate that the extraction method significantly influences phytochemical recovery and antioxidant performance, with ethanol maceration producing extracts with greater antioxidant potential.

Keywords: *Cajanus cajan*; extraction methods; flavonoid content; phenolic content; antioxidant activity.

INTRODUCTION

Plant-derived bioactive compounds have been widely investigated due to their potential applications in food preservation, pharmaceutical formulations, and nutraceutical products. Among these compounds, phenolics and flavonoids are particularly important due to their ability to scavenge free radicals and reduce oxidative stress associated with various chronic diseases. Consequently, plant-based antioxidants have attracted considerable attention in recent years as safer alternatives to synthetic antioxidants (Haji, Teka, Bereka, et al., 2024). *Cajanus cajan* (pigeon pea or kacang gude) is a leguminous plant belonging to the Fabaceae family and is widely cultivated in tropical and subtropical regions. The seeds are commonly used as food and contain various phytochemicals, including phenolic compounds, flavonoids, and tannins, which may contribute to antioxidant activity (Doloking et al., 2022). Despite their nutritional importance, the phytochemical profile and antioxidant potential of *C. cajan* seeds remain underexplored compared with those of other legumes such as soybean and mung bean.

The extraction of plant bioactive compounds is strongly influenced by extraction conditions, particularly solvent polarity and extraction technique. Solvents such as ethanol and water differ in polarity, which affects the solubility and recovery of phenolic compounds from plant matrices (Riza Marjoni et al., 2023). Conventional extraction techniques, such as maceration and water soaking, are still widely used due to their simplicity and cost-effectiveness; however, variations in extraction time and solvent may affect the yield and composition of extracted phytochemicals (Xiang et al., 2024). In addition to extraction efficiency, the characterization of plant extracts is essential to understanding their chemical properties. Fourier-transform infrared (FTIR) spectroscopy is commonly used as a rapid and non-destructive method for identifying functional groups in complex plant extracts (Kurniawati et al., 2024). When combined with quantitative assays such as total flavonoid content (TFC), total phenolic content (TPC), and antioxidant activity, FTIR analysis can provide comprehensive insights into the relationship between

extraction conditions and the chemical composition of plant-derived antioxidants (Turay & Kargbo, 2025).

Although several studies have reported the presence of antioxidant compounds in *C. cajan*, comprehensive investigations that integrate solvent variation, extraction technique, spectroscopic characterization, and antioxidant evaluation remain limited. Therefore, this study aims to evaluate the effect of different extraction solvents and techniques on the phytochemical profile, FTIR spectral characteristics, total flavonoid content, total phenolic content, and antioxidant activity of *Cajanus cajan* seed extract, as well as to examine the relationship between these bioactive compounds and their antioxidant potential.

MATERIALS AND METHODS

The overall experimental design is illustrated in Figure 1.

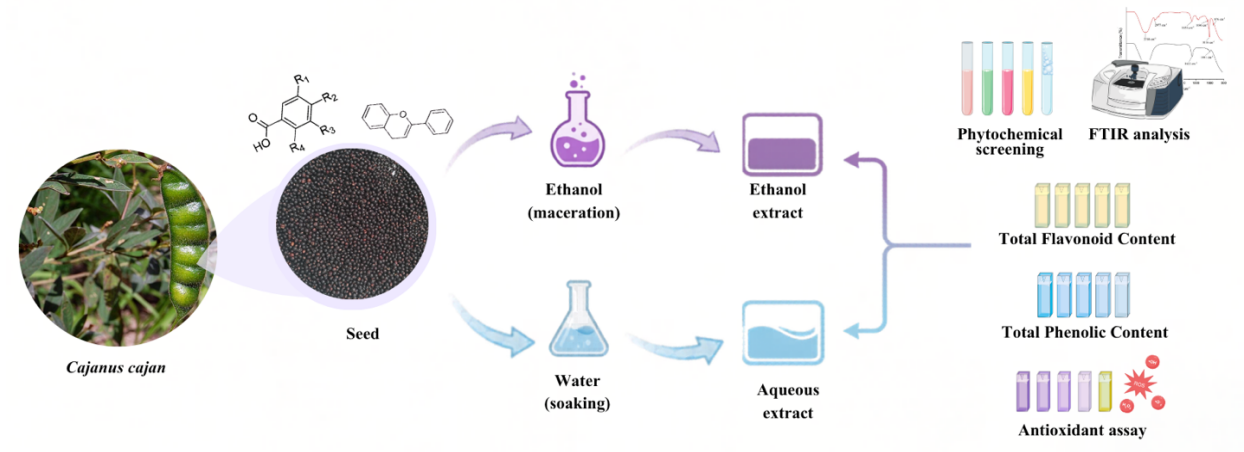


Figure 1. Experimental design of *Cajanus cajan* seed extraction and analysis.

Chemicals and equipments

The materials used in this study included *Cajanus cajan* seeds (locally known as kacang gude or lebui), distilled water, ethanol (96%, Merck), methanol (96%, Merck), aluminum chloride ($AlCl_3$, Sigma-Aldrich), hydrochloric acid (HCl, Merck), 2,2-diphenyl-1-picrylhydrazyl (DPPH, Sigma-Aldrich), Folin-Ciocalteu reagent (Merck), gallic acid (Sigma-Aldrich), magnesium powder (Mg, Merck), ferric chloride ($FeCl_3$, Merck), potassium acetate (CH_3COOK, Sigma-Aldrich), sodium carbonate (Na_2CO_3, Sigma-Aldrich), quercetin (Sigma-Aldrich), Liebermann-Burchard reagent (Sigma-Aldrich), Dragendorff's reagent (Sigma-Aldrich). The instruments used in this study included a UV-VIS spectrophotometer (Shimadzu UV-1900i), a Fourier-transform infrared (FTIR) Spectrometer (PerkinElmer Spectrum Two IR), a rotary evaporator (IKA RV10 Digital V), an oven (Memmert UN110), a hotplate (Thermo Scientific), filter paper (Whatman®), micropipettes (Eppendorf), and laboratory glassware.

Preparation of *Cajanus cajan*

Cajanus cajan seeds were selected and weighed to obtain approximately 500 g of sample. The seeds were thoroughly washed under running water to remove surface impurities and gently dried with tissue paper. The cleaned seeds were then spread evenly on aluminum trays and oven-dried at 50°C for 8 h to remove residual moisture. The dried material was coarsely ground in a household blender for 1–2 minutes and then sieved through a 10-mesh sieve to ensure a uniform particle size. The obtained powder was transferred into airtight, labeled containers and kept until further use.

Extraction of *Cajanus cajan*

For ethanol extraction, 50 g of dried *Cajanus cajan* powder was macerated with 250 mL of 96% ethanol (solid-to-solvent ratio 1:5, w/v) and stirred at 200 rpm for 24 h at room temperature. The extraction was repeated three times, and the combined filtrates were collected and stored in a closed container. This extract was referred to as the ethanol extract. Then, for the soaking extraction, 50 g of seeds were soaked in 200 mL of distilled water at room temperature for 3 days. The solvent was replaced every 24 h to maintain extraction efficiency. After extraction, the mixture was blended and filtered to obtain the aqueous extract. Both extracts were subjected to further analyses.

Phytochemical Screening

All samples were analyzed to identify the presence of flavonoids, alkaloids, terpenoids/steroids, saponins, and tannins, following the protocols established in previous studies (Fecker et al., 2020).

FTIR Analysis

Functional groups of the extracts were analyzed using attenuated total reflectance (ATR) in the range of 4000–

400 cm^{-1}. The obtained spectra were used to identify characteristic absorption bands of major phytochemical constituents.

Total flavonoid content (TFC) analysis

The total flavonoid content was measured following the aluminum chloride colorimetric method described by previous studies with minor modifications. A stock solution of quercetin (100 ppm) was prepared by accurately weighing 10 mg of quercetin and dissolving it in 100 mL of 96% methanol. Then, the various standard solutions were prepared at serial concentrations of 1, 2, 4, 6, 8, and 10 ppm. The ethanol extract was prepared at a concentration of 25,000 ppm (250 mg dissolved in 100 mL of solvent). For the assay, 0.5 mL of extract was mixed with 1 mL of 10% (w/v) aluminum chloride solution. After 6 min, 1 mL of 1 M sodium carbonate was added, and the volume was adjusted to 5 mL with distilled water. The absorbance was measured at 436 nm using a UV-VIS spectrophotometer. A calibration curve was constructed using quercetin as the standard, and the total flavonoid content was calculated based on the linear regression equation. The results were expressed as milligrams of quercetin equivalent per gram of extract (mg QE/g) (Suryani et al., 2025).

Total phenolic content (TPC) analysis

The total phenolic content of *C. cajan* extracts was determined using the Folin–Ciocalteu method. A stock solution of gallic acid (100 ppm) was prepared by weighing 10 mg of gallic acid and dissolving it in 100 mL of 96% methanol. The standard solutions were prepared by serial dilutions of 2, 4, 6, 8, 10, 20, and 40 ppm. The extract samples were prepared at a concentration of 6,000 ppm (60 mg dissolved in 100 mL of solvent). For the assay, 0.5 mL of extract was mixed with 2.5 mL of 10% (v/v) Folin–Ciocalteu reagent and incubated for 5 min in the dark. Subsequently, 2 mL of 7.5% (w/v) sodium carbonate solution was added, and the mixture was incubated at room temperature for 30 min. The absorbance was measured at 736 nm using a UV–Vis spectrophotometer. A calibration curve was conducted using gallic acid as the standard, and the total phenolic content was calculated based on the linear regression equation. The results were expressed as milligrams of gallic acid equivalent per gram of extract (mg GAE/g) (Martono et al., 2019).

Antioxidant activity of *Cajanus cajan*

The antioxidant activity was assessed using the DPPH free radical scavenging assay according to previous research (Segoviano-León et al., 2025). A 0.1 mM DPPH solution in 96% methanol was freshly prepared. The extracts were prepared at various concentrations of 0, 20, 40, 60, 80, and 100 ppm. For the assay, 1 mL of each extract solution was mixed with 1 mL of DPPH solution and incubated in the dark for 30 min at room temperature. The absorbance was measured at 517 nm using a UV-VIS spectrophotometer. The percentage of DPPH radical scavenging activity was calculated using the following equation:

$$Inhibition\ (\%) = \frac{A_0 - A_s}{A_0} \times 100$$

Where A_0 is the absorbance of the control (without sample) and A_s is the absorbance of the sample. The IC_{50} value, defined as the concentration of extract required to inhibit 50% of DPPH radicals, was determined from the plotted graph of inhibition percentage versus sample concentration.

Data Analysis

All analytical measurements were conducted in triplicate, and the results are presented as mean ± standard deviation. Statistical analysis was performed using an independent samples t-test to compare ethanol and aqueous extract for TFC, TPC, and antioxidant activity, with significance considered at $p < 0.05$.

RESULTS AND DISCUSSION

Result

Phytochemical profile of *Cajanus cajan* seed extracts

Phytochemical screening was conducted to qualitatively determine the presence of secondary metabolite groups within the samples. Common phytochemical tests included the detection of metabolites such as alkaloids, saponins, terpenoids/steroids, tannins (phenolics), and flavonoids. The results of the phytochemical screening performed on *C. cajan* samples are presented in Figure 2.

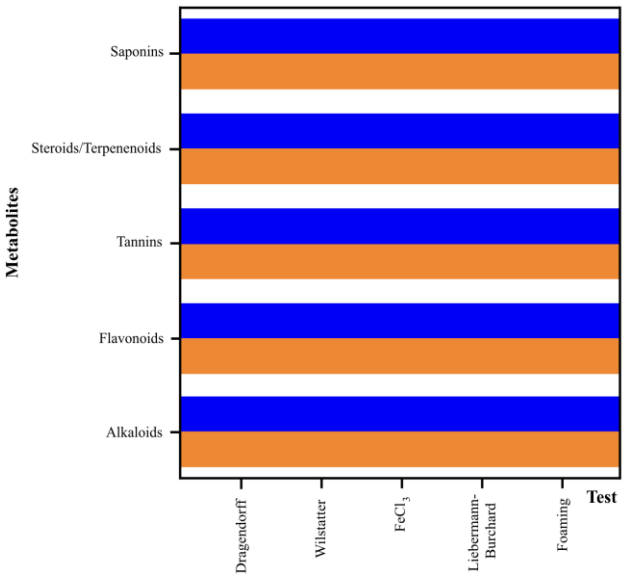


Figure 2. The phytochemical result of the presence of metabolites (blue for ethanol extract and orange for aqueous extract).

Phytochemical screening showed that both the 96% ethanolic and aqueous extracts of *Cajanus cajan* seeds

contained flavonoids, tannins, alkaloids, steroids/terpenoids, and saponins, indicating that the major secondary metabolites were successfully extracted regardless of solvent. This similarity suggests that *Cajanus cajan* seeds are naturally rich in polar to moderately polar phytochemicals that can be recovered using either ethanol or water.

FTIR analysis

The FTIR spectrum of the ethanol extract exhibited absorption peaks at 3318, 2977, 1634, 1080, and 876 cm^{-1}. Meanwhile, the aqueous extract showed absorption peaks at 3325, 1633, and 1001 cm^{-1}. The corresponding FTIR spectra of both extracts are presented in Figure 3.

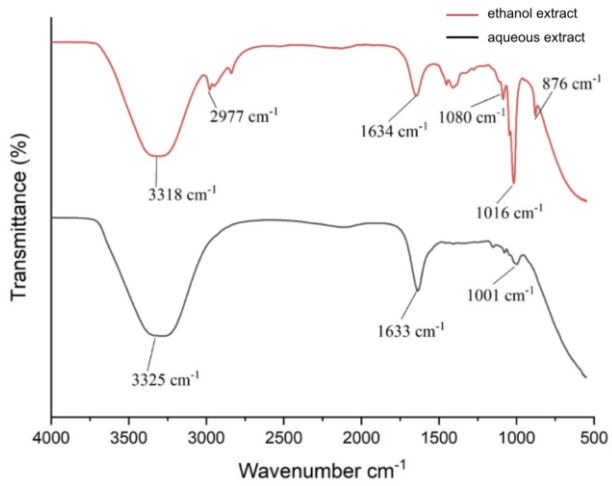


Figure 3. FTIR spectra of *Cajanus cajan* seed extract

The broad absorption band observed in the region of 3318 – 3325 cm^{-1} is attributed to OH stretching vibrations, indicating the presence of hydroxyl groups (OH), typical of phenolics and flavonoids. The peak around 1633 – 1534 cm^{-1} corresponds to C=C stretching of aromatic compounds. In the ethanol extract, the peak at 2977 cm^{-1} is associated with aliphatic C-H stretching, while the band at 1080 is related to alcohol, ether, or glycosidic groups, commonly found in flavonoid glycosides. Whereas the 876 cm^{-1} is attributed to aromatic C-H bending.

Total flavonoid content (TFC) and total phenolic content (TPC)

The total flavonoid and phenolic content of both extracts was determined from three independent replicates for each treatment. Individual replicate values are presented in Figure 4, while values and standard deviations are summarized in Table 1. The values were calculated using linear regression equations derived from standard calibration curves.

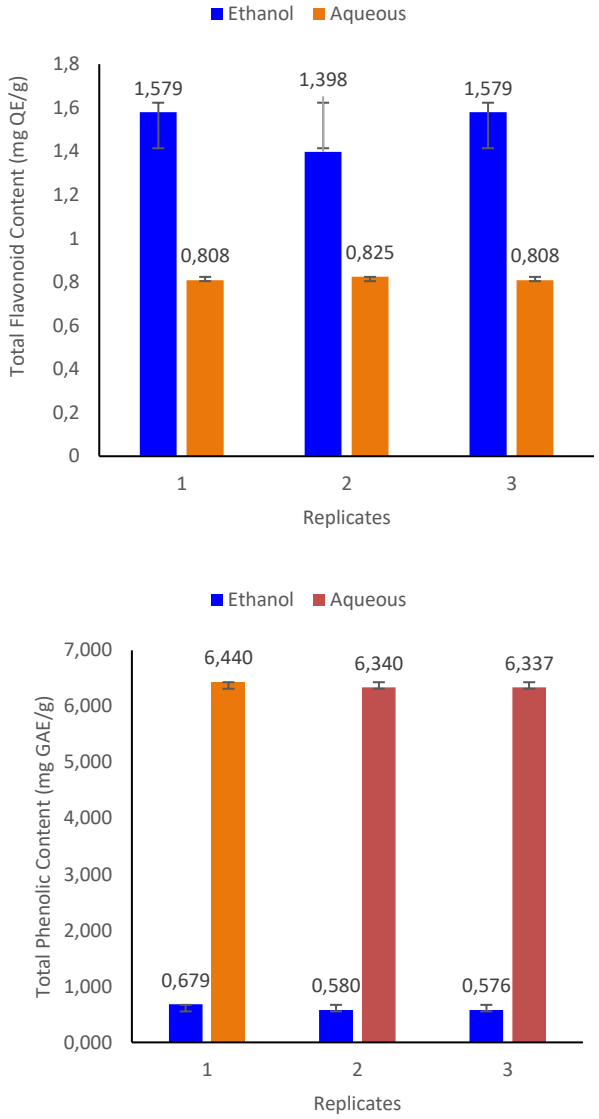


Figure 4. Replicates of TFC (left) and TPC (right) values of *Cajanus cajan* seed extract

The calibration curve for TFC was y = 0.0122x + 0.0207 with R^2 = 0.9935. Similarly, the calibration curve for TPC was y = 0.0081x + 0.0684, with R^2 = 0.9827. Based on these equations, the ethanol extract exhibited higher total flavonoid content (1.519 mg QE/g extract) than the aqueous extract (0.814 mg QE/g of extract). In contrast, the aqueous extract showed higher total phenolic content (6.373 mg GAE/g of extract) compared to the ethanol extract (0.612 mg GAE/g of extract).

Table 1. The total flavonoid and phenolic content of *Cajanus cajan* seed extract.

Sample	Total Flavonoid* (mg QE/g)	Total Phenolic** (mg GAE/g)
Ethanol extract	1.519±0.104[a]	0.612±0.058[a]
Aqueous extract	0.814±0.090[b]	6.373±0.059[b]

*The total flavonoid content is expressed in mg quercetin equivalent/gram of extract (QE/g extract). **The total phenolic content is expressed in mg gallic acid equivalent/gram of extract (QE/g extract). The TFC and TPC value followed by a different lowercase letter indicates a significant difference between groups ($p<0.05$). The difference in TFC value was based on an independent samples t-test ($p = 0.0178$). The difference in TPC value was based on an independent samples t-test ($p = 1.88 \times 10^{-7}$).

Antioxidant activity

The antioxidant activity of *Cajanus cajan* seed extracts increased with rising concentration, as shown by the percentage inhibition obtained from three independent replicates Figure 5. The antioxidant activity of the extracts was evaluated using the IC_{50} parameter, with the value shown in Table 2. The DPPH assay was employed because it offers a simple, rapid, and sensitive approach for determining antioxidant activity.

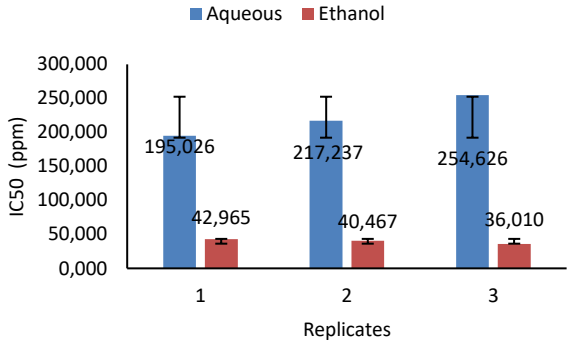


Figure 5. IC_{50} replicates of *Cajanus cajan* seed extract

The antioxidant activity of the extracts was evaluated using the IC_{50} parameter. The ethanol extract showed an IC_{50} of 39.814 ppm, classified as a very strong antioxidant, whereas the aqueous extract showed an IC_{50} of 222.296 ppm, classified as a very weak antioxidant.

Table 2. The IC_{50} value of *Cajanus cajan* extract

Sample	IC_{50} (ppm)	Category
Ethanol extract	39.814±3.523[a]	Very strong
Aqueous extract	222.296±30.120[b]	Very weak

The IC_{50} value of both extracts, followed by a different lowercase letter, indicates a significant difference between groups ($p<0.05$). The difference in IC_{50} value was based on an independent sample t-test ($p = 0.0089$).

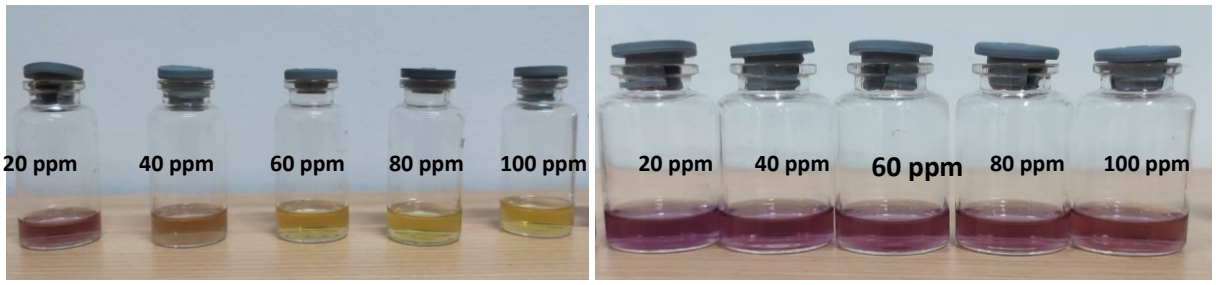


Figure 6. Color changes observed during the DPPH radical scavenging assay of ethanolic (A) and aqueous (B) extracts of *Cajanus cajan* seeds at increasing concentrations.

The ethanolic extract exhibited a progressive color change from deep purple to pale yellow as the concentration increased, indicating efficient reduction of DPPH radicals. In contrast, the aqueous extract remained predominantly purple, reflecting lower radical-scavenging activity.

Discussion

Pigeon pea (*Cajanus cajan*), locally known as "kacang gude" or "kacang lebui", is widely recognized as a potential source of natural antioxidants and bioactive compounds. The findings clearly show that the type of extraction solvent plays a central role in shaping the chemical profile and biological activity of the extracts. Rather than simply affecting yield, solvent polarity appears to determine how efficiently different classes of compounds are released from plant tissues (Bitwell et al., 2023; Ghenabzia et al., 2023; Lefebvre et al., 2021). Both extracts contained similar classes of secondary metabolites, indicating that both water and ethanol were

capable of extracting polar phytochemicals. However, differences in their relative abundance suggest that extraction efficiency was governed by solvent polarity and solvent–matrix interactions, which influence compound solubility, diffusion, and desorption from plant tissues. Ethanol, with its intermediate polarity, is capable of dissolving both moderately polar and slightly non-polar phytochemicals, whereas water preferentially extracts highly polar constituents through hydrogen bonding and dipole interactions (Bitwell et al., 2023). Previous studies have shown that *Cajanus cajan* contains various bioactive polyphenols, particularly isoflavones like genistein and biochanin A, flavones like orientin, vitexin, luteolin, and apigenin, and stilbenoids like cajanol, longistylin A, and longistylin C, which are largely responsible for the biological activities of *Cajanus cajan* (Gargi et al., 2022; Tekale et al., 2016), making them more soluble in hydroalcoholic or alcoholic solvents than in water alone. Conversely, highly polar phenolic acids, sugars, amino acids, and other hydrophilic constituents are extracted more readily by water.

FTIR analysis provided further evidence that the extraction solvent influenced the chemical composition of *Cajanus cajan.* The two extracts showed comparable functional groups associated with phenolic and flavonoid structures, although subtle differences in spectral patterns suggest variations in the proportion of oxygenated and aromatic constituents. This likely reflects differences in solvent selectivity during extraction (Dewi, 2025; Krysa et al., 2022). Both extracts exhibited hydroxyl groups (OH) at 3318 – 3325 cm^{-1}, which are essential structural features of flavonoids such as genistein, luteolin, and orientin. These functional groups indicate the presence of hydroxylated phytochemicals capable of participating in hydrogen bonding. The ethanolic extract also showed a band at 2977 $cm^{-1,}$ assigned extracts contains moderately polar bioactive compounds. Comparable peaks between 2920 and 2980 cm^{-1} have previously been observed in *C. cajan* extracts and are generally associated with methyl and methylene groups of phytochemicals, including flavonoids and other secondary metabolites (Avalos et al., 2023; Monrroy & García, 2025).

Absorption bands at 1080 cm^{-1} for ethanolic extract and 1001 cm^{-1} for aqueous extract correspond to C–O stretching vibrations of alcohols, ethers, and glycosidic linkages. Similar bands have been described in FTIR analyses of *C. cajan* and were associated with oxygenated phytochemicals, particularly flavonoid glycosides and phenolic derivatives (Monrroy & García, 2025; Pal et al., 2011). These functional groups are commonly found in compounds such as orientin and vitexin, which have previously been identified in pigeon pea (Haji, Teka, Yirga Bereka, et al., 2024; Wu et al., 2024). The aqueous extract exhibited a characteristic absorption band at 1633 cm^{-1}, corresponding to aromatic C=C stretching vibrations associated with conjugated aromatic systems and commonly observed in polyphenolic compounds, whereas the ethanolic extract displayed a peak at 876 cm^{-1}, attributed to aromatic C–H bending (Monrroy & García, 2025). These aromatic functional groups have also been reported in *C. cajan* extracts and reflect the presence of conjugated aromatic structures typical of flavonoids and stilbenoids, including genistein, biochanin A, cajanol, and longistylins (Dewi, 2025; Gargi et al., 2022; Haji, Teka, Yirga Bereka, et al., 2024).

Quantitative determination of total flavonoid content demonstrated that the extraction approach markedly influenced flavonoid recovery from *C. cajan* seeds. The ethanolic extract exhibited a markedly higher TFC value of 1.519 $\pm$ 0.104 mg QE/g than that of the aqueous extract, with a value of 0.814 $\pm$ 0.090 mg QE/g. Extraction efficiency depends not only on solvent polarity but also on solvent penetration into plant tissues, diffusion of intracellular metabolites, and the establishment of concentration gradients during mass transfer. Maceration with ethanol promotes rapid diffusion of moderately polar compounds while minimizing degradation of oxidation-sensitive phytochemicals, whereas prolonged soaking in water preferentially extracts highly polar constituents but is generally less efficient for recovering flavonoids with amphiphilic structures (Alelah et al., 2026; Nair et al., 2026).

A similar trend has been reported in previous studies on *C. cajan*, where hydroethanolic or ethanolic extracts consistently produced higher flavonoid contents than aqueous extracts. For example, 50% ethanolic seed extract contained approximately 15.13 mg QE/g, whereas the corresponding hot-water extract contained only 0.97 mg QE/g, demonstrating the superior efficiency of hydroethanolic solvents in recovering flavonoids. Likewise, ultrasound-assisted extraction of *C. cajan* leaves using 60% ethanol yielded a total flavonoid content of 30.4 mg QE/g, substantially higher than values obtained using less optimized extraction conditions (Yang et al., 2020). This finding can be attributed to the intermediate polarity of ethanol, which enables the extraction of a broader spectrum of flavonoids, including both aglycone and glycosylated forms, through enhanced solubility and mass transfer during extraction (Bitwell et al., 2023; Ghenabzia et al., 2023).

The higher flavonoid concentration observed in the ethanolic extract is also supported by the FTIR spectra. The intense O–H stretching bands detected around 3318 cm^{-1} together with aromatic C–H bending at 876 cm^{-1} indicate the presence of hydroxylated aromatic compounds characteristic of flavonoids and stilbenoids (Haji, Teka, Yirga Bereka, et al., 2024). These structural features are particularly important because hydroxyl groups serve as hydrogen donors, whereas conjugated aromatic systems stabilize the resulting phenoxyl radicals through resonance. Accordingly, the higher TFC

measured in the ethanolic extract suggests that ethanol preferentially extracted flavonoids possessing structural characteristics associated with potent antioxidant activity (Tumilaar et al., 2023). Consequently, these compounds exhibit greater affinity for hydroalcoholic or ethanolic solvents than for water alone, allowing ethanol to efficiently dissolve and diffuse these metabolites from the seed matrix (Rodríguez De Luna et al., 2020).

Interestingly, an opposite trend was observed for total phenolic content. Despite containing lower flavonoid levels, the aqueous extract exhibited a substantially higher TPC value of 6.373±0.059 mg GAE/g than the ethanolic extract with a value of 0.612±0.058 mg GAE/g. Water possesses a higher dielectric constant than ethanol and therefore exhibits greater affinity for highly polar constituents (Spange et al., 2022). Similar variability has been reported in previous studies on *Cajanus cajan*, demonstrating that TPC is strongly influenced by the (Baliyan et al., 2022) plant organ, extraction solvent, and extraction conditions. Previous studies reported that hot-water extracts of *C. cajan* seeds contained 10.93 mg GAE/g dry weight, whereas extraction with 50% ethanol increased the TPC to 23.15 mg GAE/g dry weight. The same study also showed that roots possessed considerably higher phenolic contents than seeds and leaves, indicating that both solvent polarity and plant tissue markedly affect phenolic recovery (Yang et al., 2020). Furthermore, a recent review summarized that TPC values reported for different *C. cajan* organs range from approximately 4.27 to 92.00 mg GAE/g dry weight, highlighting the substantial variation attributable to genotype, plant organ, extraction solvent, and analytical methodology (Gargi et al., 2022).

At first glance, the higher TPC observed in the aqueous extract appears inconsistent with the FTIR profile and the significantly higher flavonoid content obtained from the ethanolic extract. However, this discrepancy can largely be explained by the analytical principle of the Folin–Ciocalteu assay. Unlike flavonoid-specific colorimetric methods, the Folin–Ciocalteu assay estimates the overall reducing capacity of an extract through electron transfer reactions involving phosphomolybdic–phosphotungstic complexes rather than selectively quantifying phenolic compounds (Singleton et al., 1999). Because water preferentially extracts highly polar constituents, the elevated TPC observed in the aqueous extract likely reflects the combined contribution of genuine phenolic compounds together with other hydrophilic reducing molecules. This explanation is consistent with recent reviews demonstrating that solvent polarity governs the selectivity of phytochemical extraction, with water favoring highly polar phenolic acids and other reducing compounds, whereas ethanol more efficiently extracts moderately polar flavonoids and related polyphenols (Gargi et al., 2022). Because prolonged aqueous soaking favors the extraction of these highly polar compounds, the significantly higher TPC observed in the aqueous extract likely reflects the combined contribution of phenolic and non-phenolic reducing substances rather than flavonoids alone.

The antioxidant activity of *Cajanus cajan* seeds was evaluated using DPPH assays, which operate both the single-electron transfer (SET) and hydrogen-electron transfer (HET) mechanisms (Hassanpour & Doroudi, 2023). The antioxidant activity of *C. cajan* seed extracts was strongly influenced by the extraction solvent. The ethanolic extract exhibited a markedly lower DPPH IC_{50} value of 39.814 ppm than the aqueous extract IC_{50} value of 222.296 ppm, indicating substantially stronger radical-scavenging activity. According to the DPPH antioxidant activity classification, extracts with IC_{50} values below 50 ppm are considered to possess very strong antioxidant activity, whereas those with IC_{50} values above 200 ppm exhibit very weak antioxidant activity. Accordingly, the ethanolic extract in the present study demonstrated very strong antioxidant capacity, while the aqueous extract showed considerably weaker radical-scavenging activity (Gulcin, 2025).

Based on Fig. 6, the visual changes observed during the DPPH assay further supported the quantitative antioxidant results. As the concentration of the ethanolic extract increased, the reaction mixture gradually changed from deep purple to pale yellow, indicating progressive reduction of DPPH radicals by antioxidant compounds present in the extract. In contrast, the aqueous extract remained predominantly purple even at higher concentrations, suggesting limited radical-scavenging capacity. These visual observations are in good agreement with the measured IC_{50} values, in which the ethanolic extract exhibited significantly stronger antioxidant activity than the aqueous extract (Baliyan et al., 2022). The color transition is attributed to the reduction of the stable purple DPPH radical (DPPH•) into its non-radical hydrazine form (DPPH-H) following hydrogen atom or electron donation from antioxidant molecules. Extracts containing higher concentrations of structurally active flavonoids accelerate this reduction process, resulting in a greater decrease in absorbance at 517 nm accompanied by a visible loss of the characteristic purple color. Therefore, the rapid discoloration observed in the ethanolic extract provides qualitative evidence supporting its superior radical-scavenging efficiency. Similar color changes have been widely reported for flavonoid-rich plant extracts evaluated using the DPPH assay (Baliyan et al., 2022).

The present results are consistent with previous studies demonstrating that solvent polarity plays a crucial role in determining the antioxidant potential of *C. cajan*. Fu et al. (2023) reported that conventional ethanolic extracts of *C. cajan* leaves exhibited DPPH radical-scavenging activity with an IC_{50} of 520 ppm, whereas the encapsulated polyphenol fraction displayed enhanced antioxidant activity due to improved polyphenol recovery

(Fu et al., 2023). Likewise, Nan et al. reported that ethanolic leaf extracts of *Cajanus cajan* showed stronger DPPH radical-scavenging activity (IC_{50} = 242.01 ppm) than aqueous extracts (IC_{50} = 404.91 ppm), confirming that ethanol preferentially extracts bioactive antioxidant constituents from *C. cajan* (Nan et al., 2009). Although the absolute IC_{50} values reported in those studies differ from the present investigation because of differences in plant organs, solvent concentration, extraction procedures, and experimental conditions, the overall trend consistently demonstrates the superiority of ethanolic extraction for enhancing antioxidant activity (Bitwell et al., 2023; Nguyen et al., 2024).

CONCLUSIONS

This study demonstrates that the extraction solvent plays a decisive role in determining the phytochemical composition and antioxidant activity of *Cajanus cajan* seed extracts. Although both ethanol and aqueous extraction yield similar classes of secondary metabolites, their quantitative profiles and biological activities differed significantly. Ethanol extraction produced a flavonoid-rich extract with stronger antioxidant activity, while aqueous extraction favored the recovery of higher total phenolic content but showed weaker radical scavenging capacity. These findings indicate that antioxidant performance is more closely associated with flavonoid composition and compound selectivity rather than total phenolic content alone. Ethanol is a more effective solvent for extraction methods obtaining bioactive compounds with higher antioxidant potential from *Cajanus cajan* seeds.

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Authors' Contributions: Yuli Kusuma Dewi was responsible for conceptualization, formal analysis, and data curation of the study. The research methodology was developed collaboratively by Yuli Kusuma Dewi and Novia Suryani. Project administration and field investigations were carried out by Novia Suryani and Saza Amalia. Laboratory investigations were conducted by Yuli Kusuma Dewi and Novia Suryani, while Saza Amalia and Septiana Nabila contributed to laboratory material preparation and experimental support. The original draft of the manuscript was written by Novia Suryani and Yuli Kusuma Dewi. All authors contributed to the review and editing of the manuscript and have read and approved the final version for publication.

Competing Interests: The authors declare that there are no competing interests.

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